VOLUME XXII NUMBER 6

BOTANICAL GAZETTE

DECEMBER 1896

A RUST AND LEAF CASTING OF PINE LEAVES.

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(WITH PLATES XXII AND XXIII)

INTRODUCTION.

SOMETHING over five years ago the writer briefly described a Coleosporium,[1] or rust, occurring on the leaves of *Pinus Virginiana*, the scrub or Jersey pine.[2] The rust was found in considerable abundance on many trees, and owing to its interesting habits and its effects on the host, it was made the subject of more or less extended studies during the spring, summer, and autumn of 1891. In 1892 some additional investigations were made, this work being mainly a comparative study of the anatomy and physiology of healthy and diseased trees. In 1893 and 1894 some of the previous work was repeated and additional points in regard to the life history of the fungus and its effects on the host were brought out. Last year (1895) further studies were made. In these studies special attention was given to the shedding or casting of the leaves, a phenomenon which is known to follow the attacks of a number of fungi, and which it was thought of interest, in this case at least, to explain if possible.

[1] The systematic position of this fungus may be questioned, but as this is a matter of minor importance in the present paper it may be allowed to stand provisionally with the Coleosporiums.

[2] A new pine leaf rust, Jour. Mycology 7: 44. 1891.

I am indebted to Mr. Albert F. Woods for much assistance in the later stages of the work, and to Mr. Theo. Holm for a number of the anatomical drawings, and aid in finishing my own figures.

DISTRIBUTION OF THE HOST AND PARASITE.

Pinus Virginiana is a native of the eastern United States, being found in considerable abundance from central Pennsylvania southward to middle Georgia and westward to western Kentucky and Tennessee. Under favorable conditions the tree attains a height of 20 to 30^{m}, with a trunk 25 to 75^{cm} in diameter. In the District of Columbia and country immediately adjacent this is the most common species of pine, many of the old fields and waste grounds being overgrown with trees ranging in height from 3 to 5^{m}. It is on these that the Coleosporium is usually found, the parasite as a rule being especially prevalent on trees 2 to 4^{m} high.

The fungus has been found in more or less abundance in the District of Columbia, Maryland, and Virginia, and has also been collected by the writer on Lookout Mountain, in Tennessee, and at Asheville, N. C. No attempt has been made to collect it elsewhere, but there is no reason to doubt its occurrence wherever the host is found. Although careful examinations have been made the Coleosporium has never been seen upon any other host but the one under consideration.

APPEARANCE OF AFFECTED TREES.

During the winter the leaves of Pinus, in common with other evergreen plants, change color, the dark green fading out to a reddish yellow as the season advances.[3] As spring approaches the foliage again resumes its normal color, growing brighter as the conditions for growth become more favorable. In the early part of May, when the trees have fully recovered their normal

[3] For an account of the physiological changes involved in the winter coloring of evergreen leaves, together with a review of the literature on the subject, see G. HABERLANDT: Untersuchungen über die Winterfärbung ausdauernder Blätter, Sitzungsber. d. Acad. d. Wiss. zu Wien **72**: —. Ap. 1876.

color, those affected by the Coleosporium may be readily detected for perhaps 100^m or more by the pale yellowish hue of the leaves and the general thinness of growth. An examination of the affected trees shows that the yellowish color is due to the effects of the fungus, which is confined almost entirely to or near the tips of the needles formed the previous season. The thin appearance is owing largely to lack of leaves, those on the tree being mainly of the previous year's growth, all the others having prematurely fallen.

The changes in the host and parasite which precede the effects noted will now be discussed, attention being called first to some of the anatomical and physiological questions connected with the normal leaves, in order that what is said in regard to the effects produced by the fungus may be better understood.

ANATOMY OF THE NORMAL MATURE LEAF.

The leaves of *Pinus Virginiana* are as a rule borne in pairs. They are of a bottle-green color,[4] vary in length from 2.5 to 7.5^{cm}, and are nearly semicircular in outline, the dorsal side being curved and the ventral nearly flat. Cross sections of numerous leaves from trees grown under varying conditions show that the needles are nearly the same size throughout, namely, from 0.60 to 0.62^{mm} in diameter. Transverse sections of the leaf show that it is divided into three well-defined regions, namely, cortical, mesophyll, and fibro-vascular (*fig. 1*).[5] The cortical region occupies the periphery, and varies in thickness and structure according to the part of the leaf under observation; the part of the tree, with respect to sun and shade, from which the leaf is taken; and the condition of the soil, as regards moisture, where the tree is grown.

The cortical region is separable into three parts: (1) the epidermis, consisting of thick-walled cells quite regular in size

[4] ROBERT RIDGEWAY, Nomenclature of colors, 1886.

[5] ARTHUR, BARNES, and COULTER: Handbook of Plant Dissection, 1886; also COULTER: Synopsis of the North American pines based upon leaf anatomy, BOT. GAZ. 11: 258, 259. 1886.

and shape, the outer walls being strongly cuticularized (*fig. 2, ep*); (2) thin-walled stereomatic cells (*fig. 2, st*); and (3) thick-walled stereomatic cells (*fig. 2, st*). Both the thin-walled and the thick-walled stereomatic cells give the characteristic reaction for lignin with thallin sulfate, phloroglucin, and anilin sulfate.[6]

Neither the thick-walled nor the thin-walled stereomatic cells occur with any great degree of regularity in the cortical region. Frequently only the thin-walled cells are present, and then again there may be a definite layer of thin-walled cells next to the epidermis proper, with scattering thick-walled cells beneath. The more common arrangement is that in which both thick-walled and thin-walled cells are indiscriminately mixed, the whole forming a ring of tissue, which gives strength and rigidity to the needles.

At various points the stereomatic tissue is interrupted by the stomata (*fig. 2*). These occur on both sides of the leaf and are generally considerably depressed, as is the case with the other pines and plants having thick epidermal parts. The stomatic furrows are filled with a waxy substance, which probably plays an important part in preventing the entrance of fungi, and also, as Wilhelm[7] has pointed out, serves as a check to transpiration.

The mesophyll region occupies the median portion of the leaf, occurring in a zone which varies in thickness from 200 to 300 μ. The cells composing it are large and thin-walled, and contain, among other things, chlorophyll, starch, and the usual contents of assimilative tissue. In cross-sections the cells are seen to be nearly polygonal in shape and joined more or less closely in rows arranged at right angles to the cortical region. The number of rows vary according to the conditions under which the leaf is grown, those in intense light having, as Stahl;

[6] BEHRENS: Tabellen zum Gebrauch bei mikroskopischen Arbeiten, 1892.

[7] Ueber eine Eigenthümlichkeit der Spaltöffnungen bei Coniferen, Ber. d. deutsch. Bot. Ges. **1**: 325. 1883.

[8] Ueber den Einfluss der Lichtintensität auf Structur und Anordnung des Assimilationsparenchyms, Bot. Zeit. **38**: 868. 1880.

has shown, a greater proportion of assimilative tissue than those grown in the shade. The cells of the tissue under consideration are, in almost every case, provided with peculiar infoldings (*fig. 3*), hence the name *Armpallisadenzellen*, adopted by Haberlandt, Strasburger, and others.

In longitudinal sections the rows of mesophyll cells are seen to be separated by more or less space, which often extends without interruption from the cortical to the fibro-vascular region (*fig. 4*). The infoldings are not easily distinguishable in sections of this kind, but are usually seen as apparent partitions, and in some cases do not appear at all.

Within the mesophyll region and completely surrounded by the cells composing it occur the two resin ducts. The ducts are nearly opposite each other, being placed about equidistant from the radius of the leaf. They are cylindrical, completely inclosed by a sheath of thick-walled stereomatic cells arranged in a single row, and are lined with thin-walled epithelium (*fig. 5*). The central portion of the leaf is occupied by the fibro-vascular region. Separating this from the mesophyll is a closed sheath, the endodermis of Oudemans.[9] This consists of a single row of more or less suberized, elongated, thin-walled cells, closely joined at the horizontal or oblique ends (*fig. 6, end*). The walls also contain lignin, as shown by staining with indol.

Within the endodermis occur the mestome bundles (two in number), tracheids, and fundamental tissue, the latter consisting of thin-walled parenchymatous cells. The orientation of the bundles is normal, *i. e.*, the hadrome is directed toward the center and the leptome toward the periphery (*figs. 1* and *7*).

LIFE OF NORMAL LEAVES.

The length of time the leaves live varies according to a number of conditions, the most important being the age and size of the tree and cold and drouth. On trees 15 to 20^m high, growing in good soil, leaves may remain on three, four, and even five

[9] Ueber den Sitz der Luftwurzeln der Orchideen, Abhandl. d. Acad. Amsterdam, Math. Phys. Klass. **9**: —. 1861.

years. On comparatively young trees, 1 to 5^m high, the leaves may fall the second year. As a rule the average length of life may be placed at two years, that is, the leaves fall the summer or autumn of the second year. The time of falling, however, is not constant, as in some cases it may occur in the spring.

Numerous physiological changes take place during the period of from three to four weeks preceding the actual fall of the needles. First, the contents of the mesophyll cells gradually disappear, this being accompanied by a change in the color of the needle from green to reddish yellow. With the disappearance of the chlorophyll bodies, large oil globules appear in the mesophyll cells, and at the same time the walls of the latter become considerably thickened. Not until a week or ten days before the pair of needles fall is it possible to make out clearly the separative layer, the formation of which does not materially differ from that which takes place in most of the ordinary deciduous plants. In case of high wind or rain the leaves are frequently blown or knocked off before the separative layer is fully developed. Usually they remain on, however, until the process is fully completed, after which they dry up and fall from their own weight.

DEVELOPMENT OF THE FUNGUS AND ITS EFFECTS ON THE TREE.

An examination of an affected tree early in April shows on the needles of the previous year's growth pale yellowish bands, which vary in width from 2 to 4^{mm}. The discolorations seldom if ever extend to the extreme tip of the needle, this point as a rule remaining normal in color up to a certain time, despite the action of the fungus on the tissues below. Close examination of the diseased areas at this time reveals numerous brown and yellow pustules, which are only slightly elevated above the surface of the leaf (*figs. 8* and *9*). The pustules are the sori of the fungus, and mark the point where the spores will eventually break through. The sori vary in width from 0.2 to 0.5^{mm}, and are from 0.2 to 1^{mm} long. As the season advances the sori increase in size, many of them coalescing and forming long

bands parallel to and including, of course, the rows of stomata. The color of the diseased part of the leaf and the sori also becomes brighter yellow. By the middle of May, if the season is a normal one, the sori reach their full development, the color at this time being bright orange. As long as the weather is dry there is little change in the sori, but at the first rain or fog they rupture the cortical tissue, forming long, bright orange red, waxy, or granular elevations, 0.5 to 1^{mm} high. If the weather continues wet the sori retain their bright color, but upon close examination they are seen to be overrun with minute cobweb-like threads. Lack of rain causes these threads to disappear, but for a time at least the sori lose none of their characteristic color, size, or shape. Rain or fog will again cause the sori to swell, to become granular and waxy, and to show the growth of colorless threads, as already described. This alternate drying up and breaking out of the sori may continue two and sometimes three weeks, or it may continue but a few days, the length of time varying with climatic conditions and different trees. In case of a spell of a week or more of rainy weather the sorus will often complete its development and collapse completely as soon as the sun comes out for a few hours. Occasional rains, followed by several days of fair weather, prolong the life of the sorus in all cases.

Under ordinary conditions of humidity the sori for the most part complete their development by the middle of June, or about one month after they first break through. The diseased portion of the leaf at this time assumes a brownish hue and becomes more or less shrunken. The sori also turn brown, collapse, and soon dry up entirely. The tissues below and immediately adjoining the diseased areas now begin to turn yellow, this discoloration gradually extending downward until the whole leaf is involved.

If both needles are affected by the fungus both change simultaneously from the normal green to yellow. If, however, only one of the needles is affected the change extends from the tip downward on the affected one and from the base upward on

the other. From yellow there is a gradual change to brownish red, the tissues in the meantime becoming considerably shrunken. Finally the pair of needles fall, this often being hastened by rain, wind, or any sudden jarring or shaking of the tree. In from three to six weeks after the fungus breaks through the tissues most of the leaves have fallen. Trees badly affected are the first to shed their leaves, defoliation being very rapid and often completed before the last-formed needles have attained full size.

MICROSCOPIC STUDIES OF THE FUNGUS.

Turning our attention to the fungus itself and the manner in which it produces the changes described, a microscopic study of transverse and longitudinal sections through the pale yellow spots found early in April shows, growing between the cells composing the mesophyll region, a coarse, colorless, septate mycelium, containing numerous granules and vacuoles (*figs. 10* and *11*). The mycelium is confined wholly to the intercellular spaces of the mesophyll region. It never penetrates the walls of the mesophyll cells, but in many cases adheres very closely to the latter, producing changes in the cell contents which will be described as we proceed. Wherever it comes in contact with the cells composing the endodermis or the resin ducts it is effectually turned aside (*fig. 18*). A comparison of the diseased and healthy tissues at this time will show no essential difference so far as thickness and size of cells are concerned. In unstained sections the entire cortical and fibro-vascular regions, as well as the resin ducts, appear intact. The contents of the mesophyll cells of the diseased leaf, however, are seen to be disorganized. The chlorophyll bodies have disappeared and in their place many large oil globules are seen. In the healthy cells the chlorophyll bodies are numerous and distinct, while there are few or no oil globules. In the cells around some of the stomata of the diseased leaf the contents are not only disorganized, but are yellow and partially opaque. The V-shaped cell below the stoma is usually not involved, but those around, and especially below, show the change in a marked manner.

At certain points, which do not seem limited to any particular region, the mycelium pushes its way toward the cortical region, and between the latter and the mesophyll cells it begins to form a sorus or spore bed (*figs. 10* and *11*). This is effected by a continued interweaving of the mycelium, which at the same time becomes more septate and brown or orange yellow. As the spore beds increase in size the cortical region is pushed upward, thus giving rise to the pustules already described. With increasing age the spore beds become more compact, so that by May 10 to 15, or just before they break the tissue, they show, beneath the cortical tissue, compact rows of rounded septate bodies (*fig. 11*), the sporophores.

The sporophores are formed in the following manner: The hyphæ, which push toward the surface, begin to enlarge at their tips (*fig. 12*). Soon a septum forms just below the swollen portion, the result being the formation of a more or less rounded cell. By successive abjunction other cells are formed, until the sporophores appear in compact rows, as already described. When mature and the proper conditions of moisture are present the upper cell of each sporophore gives rise to a mass of gelatinous substance, which has no very definite structure, so far as can be ascertained from a study of transverse sections (*fig. 13*). It is the sudden development of these gelatinous masses that ruptures the cortical tissue, thus bringing the sorus into direct contact with air, moisture, and more intense light. In from three to four hours after the cortical tissues are ruptured, cylindrical bodies, which later develop into teleutospores, begin to shape themselves out of the mass of gelatinous material (*fig. 14*). These bodies soon begin to turn yellow and in a short time their walls may be definitely made out. The outer walls of each teleutospore consist of an enormously thickened, colorless, gelatinous membrane, and it is the massing of these membranes that gives to the young sori the characteristics already described. At this time it is very difficult to make out the transverse walls separating the teleutospores. Treatment with potash solution and with nitric acid, however, brings these out distinctly.

In from four to ten hours after the sorus breaks the cortical tissues the teleutospores attain full size. By this time the gelatinous membrane has mostly disappeared, and consequently the contents and septum separating each teleutospore is plainly apparent.

The teleutospores are arranged in rows of from two to four, one above the other. They do not separate, however, from each other, and it is with some difficulty that they are removed from their attachment in the sorus. When fully mature the compound teleutospores vary in length from 75 to 150μ and in diameter from 18 to 30μ.

The spores begin to germinate as soon as mature, the process taking place only in water or saturated air. The first evidence of germination is a small protuberance, which may appear at the apex or at any of the septa. Usually the topmost cell germinates first and the others follow in the order of their arrangement (*fig. 14*). The protuberance rapidly elongates into a tube, which may grow to a length several times that of the compound spore, or may remain quite short. The length, however, depends largely on the amount of moisture present, the tube attaining its greatest length if the spore is in water and its shortest if in moist air. The contents of the spore flow out into the tube, the greater portion being near the tip and the rest more or less in groups throughout its entire length. The vacuoles in the cell flow out with the contents and may usually be found at the upper extremity of the tube.

When the tube attains its full length, this, as already indicated, depending in large part on the amount of moisture present, it begins to swell at the tip (*fig. 15*). The swelling rapidly increases in size and at the same time the walls directly below it become more or less constricted. At this point a septum is formed, this process usually lasting not more than twenty-five or thirty minutes. The body thus cut off by the septum is the sporidium, which now begins to increase in size and assumes various shapes. Occasionally the sporidium will send out a germ tube while still attached to the supporting promycelium.

Usually, however, the sporidium separates from the promycelium in about thirty or forty minutes after the partition forms, and then almost immediately begins to germinate by sending out a short, thick germ tube. All or nearly all the contents of the sporidium flow into the germ tube. Occasionally this tube grows out to a considerable length, cutting off a secondary sporidium (*fig. 16*) upon its free end in much the same way that the original was formed.

After the spore germinates the walls remain turgescent for a short time, and then collapse and dry up, the spore in the meantime becoming perfectly colorless. The entire contents of the cell seem to be used up in the formation of the promycelium and the sporidium, and if this is not the case the formation of secondary sporidia goes on until there is no protoplasm left.

Germination as here described takes place in nature only when the leaves are wet, the spores themselves seldom if ever escaping from the sorus. The sporidia, however, when cut off from the promycelia either fall or are washed down to the leaves below or to the ground, where they may be found in great abundance after a damp or foggy night. In all infection experiments, which will be referred to later, the sporidia were obtained in abundance by placing sterilized Petri dishes under the diseased branches confined in a moist chamber. After several hours an abundance of sporidia may be obtained from the dishes.

INFECTION OF THE HOST.

On closely examining the diseased trees the sporidia may be found on the young leaves, which at this time are just beginning to show their tips. The usual place for lodgment is between the tips of the two young needles, which at the time the spores are forming project about 0.33^{cm} above the sheath which incloses them. The needles are slightly spread open, thus allowing a small drop of water to be retained. The water that collects in this way drips from the old leaves above, upon which the spores and sporidia are being formed. In dropping down it carries the sporidia with it, depositing the latter in a

position where they may, under the very best conditions, germinate and infect the young needles.

For several years the time of the appearance of the spores and young leaves has been watched, and in every case a most remarkable similarity in dates has been observed. If the leaves are late or early in coming out the spores will be correspondingly late or early. This is exceedingly important in the economy of the fungus, for it has been proved by successive infections that a difference of but two days in the age of the leaf will enable it to resist the fungus. Infection, in other words, must take place when the parts of the needles are from 0.33 to 0.66^{cm} long (*fig. 19 a*, too old; *b*, proper age), otherwise the cortical region will be developed to such an extent that the germ tubes from the sporidia cannot force their way through.[10]

The needles are infected wholly by means of the germ tubes boring through the cortical tissue before the stereomatic, thick-walled cells have formed. These tubes make their way to the mesophyll region, where they immediately begin to lengthen, in eight or ten days assuming all the characteristics of the mycelium already described. The orifice, however, through which the germ tube enters the leaf, soon disappears, leaving no trace of the manner in which the fungus reaches the mesophyll region. In about three months, or by the middle of August, the fungus has developed to such an extent that its presence may be determined by the condition of the tips of the needles. Pale yellowish spots may be seen at these parts, and microscopic examination of the tissue reveals the mycelium growing in all directions between the cells of the mesophyll region. As the season advances the spots or bands become more prominent, and by the middle of November, or even earlier, pustules, indicating the formation of sori, begin to appear. By the first of April, or ten

[10] For the sake of brevity the details of the experiments are omitted. Briefly, in this case one hundred pine branches were bagged with paper bags, the work being carried on for two successive years. From time to time a bag was removed and the young leaves received a small drop of water containing germinating sporidia, after which the leaves were marked and the bag replaced. About 75 per cent. of the inoculations made when the leaves were the proper age were successful.

months after infection, the fungus is found in the condition already described. A month or six weeks later it again produces spores, thus completing its cycle of development in one year.

CASTING OF THE LEAVES AND ITS CAUSE.

As already pointed out, we have in the case of the *Coleosporium* a fungus producing first purely local injuries, which later result in the death and casting of the leaves. The phenomena involved in this process may now be considered. An examination of the diseased leaves soon after the sori collapse shows that the conductive tissue has not been injured at all, the endodermis being intact and the fibro-vascular bundles abundantly able to conduct water. That they do this is evident from the fact that, even though the mesophyll invaded by the fungus be brown and shriveled, the tip of the leaf above this part remains green for a considerable time. In the tissue where the fungus has been at work there are large rifts which extend unbrokenly to the endodermis. The cell contents of the mesophyll region immediately below the part attacked by the Coleosporium, and where there is no mycelium at all, are broken up, showing that changes are taking place which are not directly attributable to the fungus. These changes are manifested externally by the yellowing, already described, which gradually extends downward until both needles are involved. Even before this the separative layer begins to form and in a week or ten days more the pair of needles fall. Of course, the fungus itself is the primary cause of the leaf fall, but that it acts only as a power to set other causes at work was early shown by cutting off the affected portions of the leaves. In every instance this treatment caused the leaves to remain on the tree and perform their functions in a normal manner until death came from old age. In all cases, however, it was found necessary to make the cuts through normal green tissues, *i. e.*, below the point where the yellowing showed. If only one needle was allowed to become partly yellow and the cut was made through the discolored por-

tion the changes eventually leading to the fall of the leaf were not checked at all.

It was thought that possibly the fungus by its action might have produced chemical changes in the cell contents and that these changes alone were sufficient to produce the effects described. It was reasoned, however, that if there was anything in the nature of a ferment present it should be capable of propagating itself when brought in contact with the cells of healthy pine leaves. To obtain a definite answer to this question a series of experiments were made, which need not be entered upon in detail here. Suffice it to say that after more than two hundred trials with juices from diseased leaves rubbed on broken and unbroken tissues of healthy foliage and brought into contact with the healthy cells in other ways, not a single case of leaf casting or leaf yellowing occurred.

It is unnecessary to go over the ground which led to the belief that the changes, as already described, were due to excessive loss of water. The large rifts in the cortical and mesophyll regions, the uninjured conductive tissue, and the fact that no cork layer, cutting off the injured portion of the leaf, was formed, all pointed to a derangement of the water supply.

To obtain information upon this matter a number of experiments were made, some of which will now be described. The first experiments were with cobalt paper[11] to determine the relative evaporation of water from the parts affected by the fungus and the uninjured portions. The tests were made before and after the fungus had broken through, the usual method being to fasten strips of dry paper to the leaves by means of glass slides. It was found that before the fungus broke through the diseased areas lost less water than the healthy portion of the leaf. This was due to the permanent closing of the stomata over the diseased areas, owing to the action of the fungus on the adjacent tissue. The fact that the diseased spots lost less water than healthy portions of the leaf explains why such spots remain

[11] For a discussion of the cobalt method, see STAHL, Bot. Zeit **52**: 117–145. 1894; reviewed in BOT. GAZ. **21**: 26–33. 1895.

green longer than other parts when a branch is cut from the tree and allowed to die through lack of water.

As soon as the Coleosporium has ruptured the cortical tissues evaporation rapidly increases, until it exceeds that from a corresponding area of healthy tissue. This was proved by the

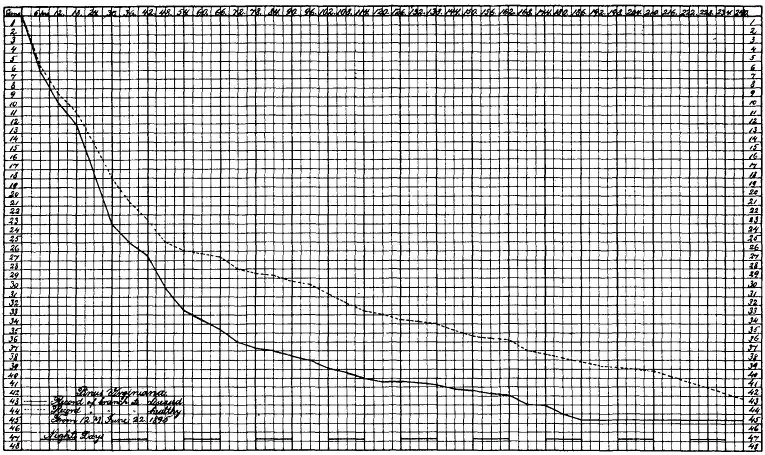

FIG. 1. Diagram showing evaporation from diseased and healthy branches.

cobalt paper test and also by the following experiments: Two branches, as nearly alike as possible, were secured and cut off under water and the cut ends sealed into a flask of normal culture solution, so that no water could escape except through the leaves. Each branch had 206 leaves. The leaves on one branch were healthy and on the other the Coleosporium had just broken through the diseased areas. Starting at 12 M. June 22, the curves in the accompanying diagram show the loss in grams in periods of six hours for ten days, as determined by a recording balance. The water supply was not as good as the dry air surrounding the leaves demanded. This lack of water was due to the accumulation of resin on the cut ends of the branches and the development in the same place of bacterial slimes. The unbroken line represents the loss from the diseased branch and the broken line the loss from the healthy

branch. It will be observed that the loss during the first forty-eight hours was rapid in both cases, namely, 25^{gm} from the healthy branch and 30 from the diseased. At the end of this time both the healthy and the diseased leaves showed an evident lack of water. This lack, however, was more marked in the diseased leaves, the tips of many of which were shrunken above the diseased area. This lack of water had caused the stomata of both the healthy and the diseased leaves to close as far as possible, thus cutting down the loss through them to a minimum. During the next forty-eight hours the healthy branch lost 4.5^{gm} while the diseased lost 8^{gm}. It was evident that from this time the leaves were able to obtain very little if any water from the stem. During the next three periods of forty-eight hours each the healthy branch lost 5, 4.5, and 2.5^{gm} respectively, while during the same periods the diseased branch lost only 3.25, 3, and 0^{gm}. The leaves of the diseased branch were completely dry at the end of 186 hours, while the healthy plant was not yet dry at the end of 240 hours, but was still losing at the rate of 1.5^{gm} per day. The more rapid drying out and death of the diseased leaves than of the healthy is therefore evident. As a further evidence of this the following experiment may be cited:

Six pairs of leaves, three of which were diseased and three healthy, were removed from the same branch and immediately cemented into a piece of cork to keep them from tipping over. Weighings were then made at given periods, with results as shown in the accompanying diagram, the broken lines representing the diseased and the unbroken the healthy leaves. It will be seen that the average loss for the healthy leaves for the first twenty-four hours was about 5^{mg}, while from the diseased leaves it was 16^{mg}. This rate of loss continued nearly constant for the healthy leaves for 174 hours, while the loss from the diseased leaves kept gradually diminishing. At the end of 120 hours the healthy had lost about 24^{mg} and the diseased 32^{mg}. The latter were now becoming quite dry, so that during the next twenty-four hours they lost an average of only 3^{mg}, while the healthy lost about 11^{mg}. At the end of this period (174 hours) the diseased leaves were air-dry, and

being hygroscopic their weight increased and diminished with the ordinary changes in atmospheric humidity. The healthy leaves continued to lose at a diminished rate and at the end of 288 hours were not yet dry.

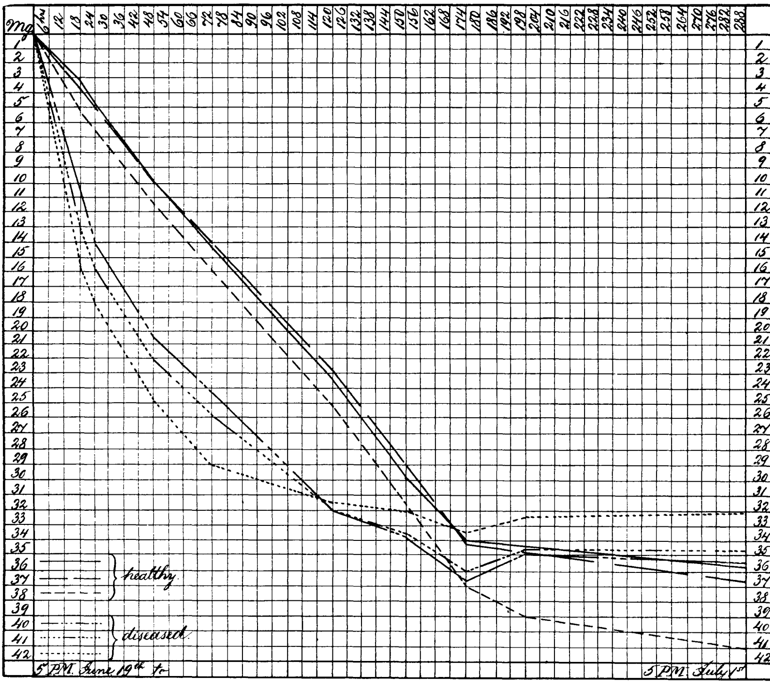

FIG. 2. Diagram showing evaporation from diseased and healthy leaves.

One additional experiment in this connection may be of interest. It was carried on during the casting of the leaves for three successive years and each time with practically the same results. Twenty-five small branches, containing about fifty pairs of leaves each, were cut during the night from a diseased tree, and immediately the cut end of each branch was pushed through a small hole in a cork and into a bottle containing water. After being fastened into the neck of the bottle, the cork and branch were sealed with paraffin, so that no water could escape except

through the leaves. Twenty-five healthy branches, taken from the same tree which furnished the diseased ones, were prepared in a similar manner. All the bottles were then brought into the laboratory and weighed twice daily for ten days. At the end of this time the branches and leaves were weighed and the amount of water evaporated per gram of dry weight was calculated. It was found that the diseased leaves evaporated approximately one-fifth more than the healthy, these results holding good through all the experiments, as will be seen by a study of the preceding diagrams.

The fact that cutting off the diseased parts of the leaves prevented them from falling has already been pointed out. It was assumed that the removal of the injured portions stopped the excessive evaporation and enabled the leaf to heal the wound made by the cut, which it could not do in case of the fungus. To prove this a number of experiments were made, of which the following is an example:

Twelve pairs of fresh leaves were selected, six pairs being diseased and six healthy. These were divided into four sets of three pairs each. Set no. 1 (healthy) had one-half inch cut from each leaf and the tips charred with red hot glass to prevent an excessive loss of water and turpentine. After charring, the tips were coated with a varnish, which prevented the entrance of fungi and slightly reduced the evaporation of water from the cut ends. Set no. 2 (diseased) was treated in the same way, the diseased ends being removed. Set no. 3 were healthy uncut leaves and set no. 4 diseased uncut leaves. All these sets were weighed at given periods and the loss in weight determined. The results are shown in the accompanying diagram.

During the first twenty-four hours the healthy cut leaves lost 9^{mg}, the diseased cut and the healthy uncut exactly 7^{mg} each, and the diseased uncut 11^{mg}. The same relative loss held good through the next forty-eight hours.

The ends of the healthy cut leaves lost water more rapidly than the ends of the diseased cut leaves, making the loss approximate more closely to that of the diseased uncut leaves. The

ends of the diseased cut leaves were more securely closed, so that they dried out only a little more rapidly than the healthy ones. This experiment shows that cutting off the diseased parts reduces evaporation to the normal amount. It appears there-

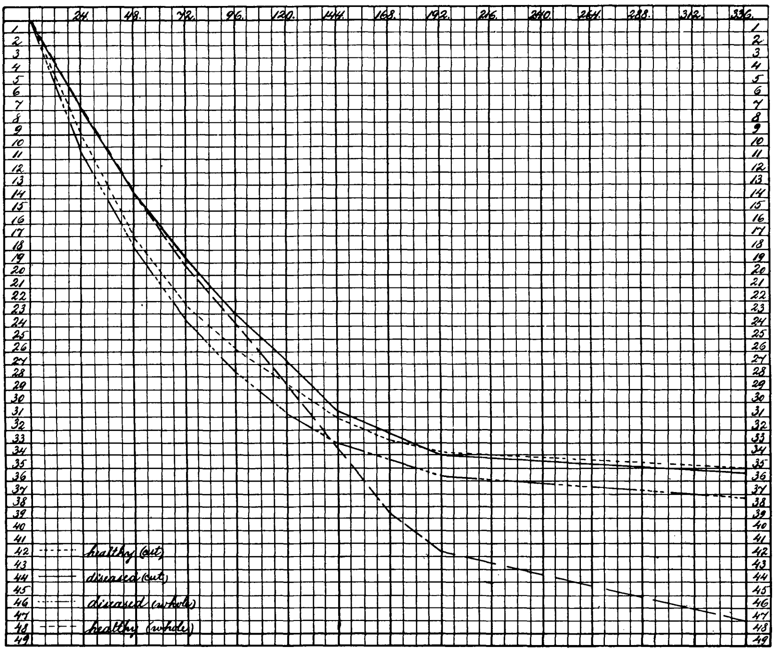

FIG. 3. Diagram showing evaporation from healthy and diseased leaves from which the tips had been cut.

fore that as soon as the fungus ruptures the cortical tissues of the leaves normal evaporation is increased about one-fifth. The physiological significance of this fact is great, as it affects the entire economy of the plant.

Without entering upon a discussion of all the processes involved it may be said that this constant loss of water from the leaf cannot be supplied by the conductive tissue. Drawing on the reserves of water in the cells is therefore necessary, and this is followed by loss of turgidity, cessation of growth, and finally

death. The strain is so gradual, however, that the changes preceding death do not differ from those which take place in a dying leaf after having lived its allotted time.

SUMMARY.

To recapitulate, the investigations here described have shown that:

(1) *Coleosporium pini* occurs abundantly in Maryland, Virginia, and the District of Columbia, attacking only *Pinus Virginiana.*

(2) It requires twelve months to complete the development of this fungus, and during a large part of that time it does not seriously interfere with the functions of its host.

(3) The fungus is disseminated by means of sporidia, which develop only during wet weather.

(4) From the leaves on which they are borne the sporidia are washed or drop to the young needles just showing their tips, which they infect. No evidence of this infection, however, is apparent for two or three months.

(5) Regardless of the season, there is a marked similarity in the time of the appearance of the sporidia and the time of the appearance of the young leaves.

(6) Before the fungus ruptures the cortical tissue evaporation from the diseased areas is less than that from healthy parts of the same leaf. This is due to the permanent closing of the stomata and may result in keeping the diseased parts alive longer than the healthy in case the leaf or branch is removed from the tree.

(7) As soon as the fungus ruptures the tissues evaporation is increased about one-fifth above the normal. In consequence of this the reserve water in the cells is gradually used up. This is followed by loss of turgidity and other physiological changes which lead to the gradual death and casting of the leaves.

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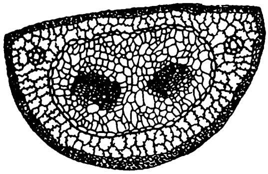

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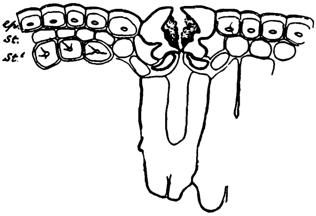

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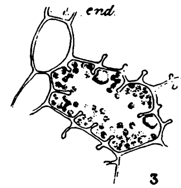

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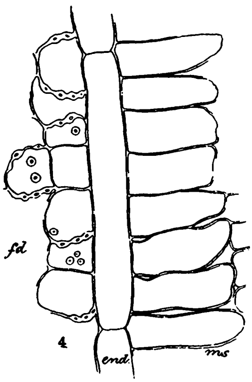

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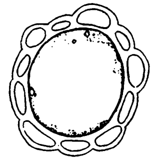

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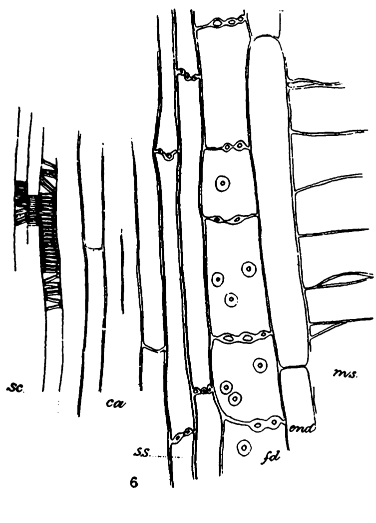

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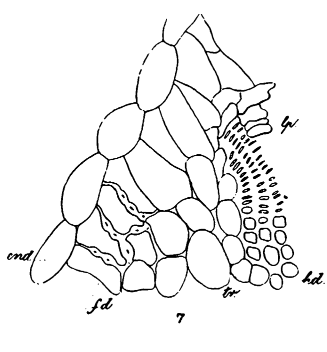
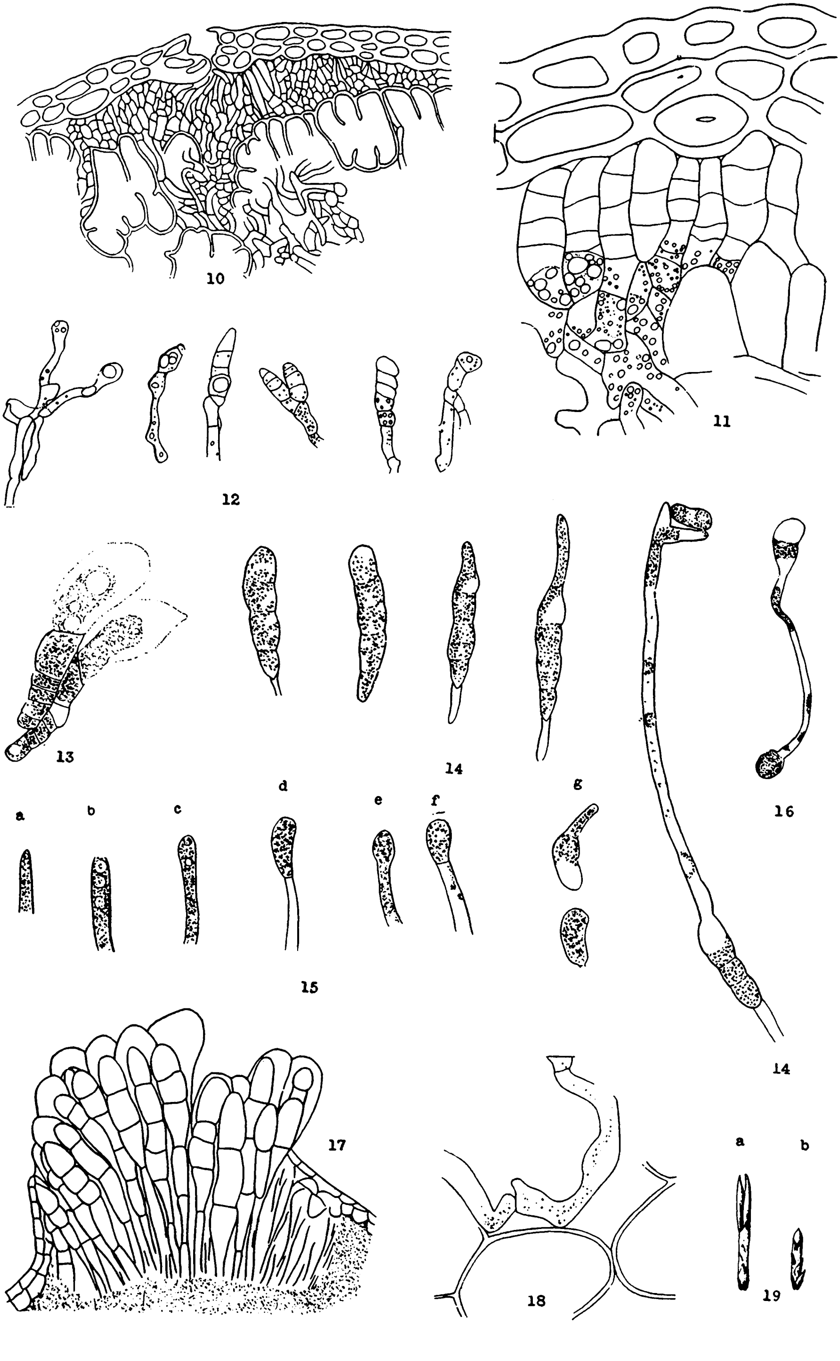


7

GALLOWAY on RUST and CASTING of PINE LEAVES.



GALLOWAY on RUST of PINE LEAVES.

EXPLANATION OF PLATES XXII AND XXIII.

FIG. 1. Cross-section of leaf of *Pinus Virginiana*, showing cortical, mesophyll, and fibrovascular regions.

FIG. 2. Cortical region and stoma; *ep*, epidermis; *st*, stereomatic thin-walled cells; *st'*, stereomatic thick-walled cells.

FIG. 3. Mesophyll cell and portion of endodermis, *end*.

FIG. 4. Longitudinal section of portion of leaf; *fd*, fundamental tissue; *end*, endodermis; *ms*, mesophyll cells.

FIG. 5. Resin duct.

FIG. 6. Longitudinal section of leaf; *ms*, mesophyll; *end*, endodermis; *fd*, fundamental tissue; *ss*, sieve tubes; *ca*, cambium; *sc*, scalariform vessels.

FIG. 7. Cross-section of a portion of leaf; *end*, endodermis; *fd*, fundamental tissue; *tr*, tracheids; *hd*, hadrome with scalariform vessels; *lp*. leptome.

FIG. 8. Branch of *Pinus Virginiana* showing Coleosporium on one year old leaves.

FIG. 9. Pair of leaves from the branch shown in *fig. 8*.

FIG. 10. Cross-section of pine leaf showing development of hyphæ.

FIG. 11. Portion of sorus of fungus showing sporophores and mycelium

FIG. 12. Various stages in the development of sporophores.

FIG. 13. Beginning of the formation of two spores.

FIG. 14. Mature spores and spores in various stages of germination.

FIG. 15. Development of sporidia, *a* to *g*.

FIG. 16. Sporidium forming a secondary sporidium.

FIG. 17. Section through a sorus showing mature spores.

FIG. 18. Mycelium turned aside by the endodermis, which it never penetrates.

FIG. 19. Two pairs of young pine leaves, *a* too old for infection, *b* the proper age for infection (both natural size).